**Review**

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# Human neuronal cells: epigenetic aspects

**Abstract:** Histone acetyltransferases (HATs) and histone deacetylases (HDACs) promote histone posttranslational modifications, which lead to an epigenetic alteration in gene expression. Aberrant regulation of HATs and HDACs in neuronal cells results in pathological consequences such as neurodegeneration. Alzheimer's disease is the most common neurodegenerative disease of the brain, which has devastating effects on patients and loved ones. The use of pan-HDAC inhibitors has shown great therapeutic promise in ameliorating neurodegenerative ailments. Recent evidence has emerged suggesting that certain deacetylases mediate neurotoxicity, whereas others provide neuroprotection. Therefore, the inhibition of certain isoforms to alleviate neurodegenerative manifestations has now become the focus of studies. In this review, we aimed to discuss and summarize some of the most recent and promising findings of HAT and HDAC functions in neurodegenerative diseases.

**Keywords:** acetylation; Alzheimer's disease; deacetylation; gene expression; neuroprotection; neurotoxicity.

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## Introduction

The term 'epigenetics', the study of how genotypes influence phenotypes *via* programmed changes during development, was first coined by Conrad Waddington (1) in 1942. More recently, epigenetics refers to the study of phenotypic alterations in gene expression without eliciting changes on the underlying DNA sequence itself (2). Intertwined within our DNA are histone proteins that are targeted by a variety of enzymes that elicit posttranslational modifications (PTMs) leading to epigenetic modifications. The most studied PTMs are histone acetylation and deacetylation. Histone acetyltransferases (HATs) have been implicated in gene expression, whereas histone deacetylases (HDACs) function in silencing gene expression (3). The precise regulation of HATs and HDACs are crucial for cellular identity and homeostasis, most particularly in neuronal cells, as they need to sustain their integrity throughout the lifetime of an individual. An aberrant epigenetic regulation in neuronal cells leads to pathological consequences such as neurodegeneration (4). Alzheimer's disease (AD) is one of the most common age-related neurodegenerative diseases of the brain, which brings about a devastating pathology and results in progressive cognitive decline and brain atrophy. Accumulating evidence suggests that neurodegenerative diseases have an epigenetic etiology (5, 6), but this is still under debate.

Currently, neurodegenerative diseases are incurable; therefore, great efforts need to be undertaken to develop therapeutic methods in ameliorating neurodegenerative diseases. The use of pan-HDAC inhibitors has provided great promise in remodeling the chromatin state to alleviate neurodegenerative manifestations (7, 8). However, reports have shown discrepancies in their functions (9, 10), which are potentially due to their nonselective nature. In recent years, HDACs have been shown to exhibit individual roles during neurodegeneration where certain isoforms are protective and others are neurotoxic. This review is aimed at discussing the most recent and promising findings associated with epigenetic mechanisms in neurodegenerative diseases.

## Neurodegenerative diseases

Neurodegeneration is the general term used to describe the progressive loss of neuronal functionality and death within the central nervous system. AD is the most common age-related neurodegenerative disease of the brain, and its prevalence in society is steadily increasing due to the aging population. Recently, the World Health Organization reported that in 2010, 35.6 million people have dementia worldwide, and this figure has been estimated to triple by the year 2050. Globally,

there are 7.7 million new cases of dementia occurring each year, 60%–70% of which can be attributed to AD. Due to the rapid increase in the prevalence of AD and dementia, there is a dramatically rising social and economical burden due to increasing numbers of unpaid caregivers and rising costs, which have been approximated at US$604 billion in 2010 (11). Due to this, AD and dementia have become a large global health issue, and great efforts need to be undertaken to deter the onset of neurodegenerative diseases.

Clinically, an individual with AD will experience sporadic episodes of memory loss and impairment of language skills and other cognitive abilities. In the advanced stages, memory loss and impairment are notably more prominent, which eventually lead to a cease in proper brain function (12). Pathological hallmarks underlying AD include extracellular deposition of amyloid β (Aβ) plaques and intracellular accumulation of neurofibrillary tangles (NFT), which account for the progressive deterioration of the brain (12). Parkinson disease (PD) is the second most prevalent neurodegenerative disease, which is characterized by intracellular inclusions of Lewy bodies and a decline in the number of dopaminergic neuronal cells within the substantia nigra pars compacta. As a result, patients with PD experience debilitating effects on motor functions including tremors, muscle rigidity, and bradykinesia (5, 13). Huntington's disease (HD) is another neurodegenerative disease affecting both cognitive and motor functions. It is an inherited autosomal dominant disorder, represented by an expansion of 36 CAG repeats on *Huntingtin*, that can manifest as early as 30 years old (13).

The pathogenesis of AD and other neurodegenerative diseases are multifactorial in nature, as both familial and sporadic types are known. Genetic predispositions are accountable for a minority of neurodegenerative diseases resulting from the mutations of risk genes, which generally lead to an early onset of neurodegeneration (5, 14). Sporadic neurodegenerative diseases appear later in life, where a variety of lifestyle choices and environmental stressors can greatly increase the risk of developing neurodegenerative diseases and dementia. For instance, dietary habits are increasingly being found to be important factors. Docosahexaenoic acid (DHA) is an essential ω-3 fatty acid that has been found to reduce the risk of developing dementia (15, 16). Moreover, caloric restriction, fruits, vegetables, and Mediterranean-like diets offer protection against neurodegeneration. In contrast, environmental exposures to certain metals and pesticides can greatly increase the likelihood of developing such ailments (5, 14).

## Epigenetics and gene expression

Age is one of the most predominant risk factors associated with neurodegenerative diseases, and deviant epigenetic regulations have been linked to this widespread phenomenon (17, 18). A great majority is known about the pathology that underlies neurodegenerative diseases, yet the etiology remains unclear. Studies featuring monozygotic twins discordant for AD provided insight into the underlying molecular mechanisms associated with neurodegeneration and further indicate that phenotypic alterations are governed by epigenetic regulations, given their genetic resemblance (19). Classically, the term 'epigenetics' refers to the mechanisms causing heritable alterations in gene expression without eliciting changes to the DNA strand itself (2).

The presence of Aβ and τ pathologies in the AD brain is inevitable. However, it still begs the question whether they are the cause or the effect of the disease. Healthy aging alone does not correlate with neuronal cell loss; however, genes associated with normal neuronal functions such as learning, memory, and signal transduction decrease with age (20). Epigenetic alterations of gene expression in the aging brain arise from DNA damage and a variety of neuronal insults, which are highly implicated during neurodegenerative maladies (20, 21). A previous study profiled and contrasted 12 633 genes of the hippocampal cornu ammonis 1 (CA1) from six sex- and age-matched AD and non-AD donors. The damaging elements were discovered to be overexpressed, such as proapoptotic and inflammatory regulators, whereas the prosurvival elements including transcriptional factors were considerably downregulated in the AD CA1 in comparison to the healthy aged controls (22). This provides a strong indication that aberrant epigenetic regulations are associated with neurodegenerative etiology.

Epigenetic regulations such as histone PTMs have been shown to diverge significantly as we age, which may be attributed to a variety of environmental factors and daily routine (23). Dementia, including AD, is not associated with healthy aging (11), and therefore, it is imperative to gain knowledge of the potential risk factors as well as the implementation of healthy lifestyle changes to deter the onset of neurodegenerative diseases. In particular, certain foods contain bioactive components that can directly influence epigenetic machinery (24). In a recent publication, the NeuroAllergy Research Laboratory demonstrated that zinc and DHA are involved in the modulation of histone H3 PTMs, including lysine (K) acetylation and dimethylation and threonine (T) phosphorylation within the M17 human neuroblastoma cells (25). The

study effectively demonstrated that the DHA treatment induced H3K9 acetylation, including K9, K36, and K79 dimethylation, which decreased when treated with zinc. Alternatively, zinc promoted K4 and K27 dimethylation and T3 phosphorylation, which were shown to decrease upon DHA treatment (25). This study provides insight into the underlying molecular mechanisms regarding dietary intake and its influence on epigenetic regulations, particularly histone PTMs in human neuronal cells. Broccoli sprouts have shown natural HDAC inhibitory effects as early as 3 h after consumption of one cup, promoting an increase in histone H3 and H4 acetylation marks in peripheral blood mononuclear cells (PBMCs) of healthy participants (26). In addition, antioxidant-rich dietary components have shown promising neuroprotective effects against neurodegenerative diseases. Observational studies have shown that daily supplementation of blueberry (27) and Concord grape (28) juice for 12 weeks enhanced cognitive performances in elderly participants displaying mild cognitive impairments (MCI) in comparison to that of the placebo-supplemented participants. Similar sets of *in vitro* studies have demonstrated that the antioxidant polyphenol anthocyanin in blueberries and strawberries can attenuate oxidative stress mediated by cytotoxic stimuli (29, 30). From this research, it can be concluded that dietary interventions could provide promising therapeutic potentials to modulate aberrant histone PTMs (4) and alleviate oxidative stress syndromes (31) associated with neurodegenerative diseases.

There are a variety of epigenetic mechanisms that function in regulating gene expression, which include DNA methylation and histone PTMs. DNA methylation involves a covalent transfer of methyl group from the *S*-adenosyl-methionine to the fifth carbon of a cytosine residue, forming a 5-methylcytosine, which is catalyzed by a family of DNA methyltransferases (32). Histone PTMs are catalyzed by a variety of enzymes that influence chromatin conformation and gene expression, such as histone methylation, phosphorylation, ubiquitination, and acetylation (33). Histone acetylation is one of the most widely studied histone PTMs, which has been highly implicated during neurodegenerative diseases (4), and will therefore be the focus of this review.

## HATs and HDACs

HATs and HDACs represent two different classes of enzymes that catalyze the acetylation and deacetylation, respectively, of K residues of histone proteins (Figure 1). The nucleosome encompasses a histone octamer, which comprises an H3 and H4 tetramer and two dimers of H2A and H2B, enveloped by 147 bp of DNA (3). Each nucleosome is held together by a varying stretch of linker DNA associated with the H1 protein (34), creating a 'beads-on-a-string' appearance (35). Through electrostatic interactions, positively charged histones and negatively charged DNA function in stabilizing nucleosomal structures and configuring chromatins (34).

The histone acetylation activity performed by HATs transfer an acetyl moiety from the coenzyme A to K residues of histone N-terminal tails. This unwinds the DNA-histone conformation, facilitating gene expression by allowing the transcription factors to interact with the DNA. In contrast to this, the deacetylation activity by HDACs results in a compressed chromatin conformation. This ultimately impedes gene expression as transcriptional processes are limited due to the DNA being inaccessible (3). In humans, HDAC1 was the first enzyme to be characterized (36). To date, there are 18 known HDAC isoforms, which are categorized into four classes. Classes 1, 2, and 4 are zinc-dependent metallo-enzymes, whereas class 3 requires nicotinamide adenine dinucleotide for their catalytic activities (37, 38). Class 1 members show a homology to yeast RPD3, which consists of HDAC1, HDAC2, HDAC3, and HDAC8, and are primarily localized within the nucleus of a cell. Class 2 consists of HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC10, which display nucleocytoplasmic shuttling and share great homology to yeast Hda1 (37, 38). This class is also subdivided into two subclasses in terms of the number of catalytic domains they possess. Class 2a includes HDAC4, HDAC5, HDAC7, and HDAC9, whereas class 2b includes HDAC6 and HDAC10, which contain one and two catalytic domains, respectively (38). However, it has recently been suggested that class 2a enzymes are not functional HDACs but rather function as acetyl lysine-binding proteins (39). HDACs belonging to class 3 are termed sirtuins, which contain SIRT1–7 and have shown high involvement in neurodegenerative diseases (40). HDAC11, the most recently characterized HDAC, is the only member of class 4, due to its differing phylogeny (41), and is shown to be the most abundant HDAC in the rat brain (42).

Together, HATs and HDACs regulate important processes that are integral to many cellular functions and survival (3, 43). The catalysis of HATs and HDACs is not solely restricted to histone proteins as they also catalyze a variety of transcriptional factors such as cAMP response element binding (CREB), whose functions have shown to be highly neuroprotective during neurodegeneration (44). In addition, they contain a variety of

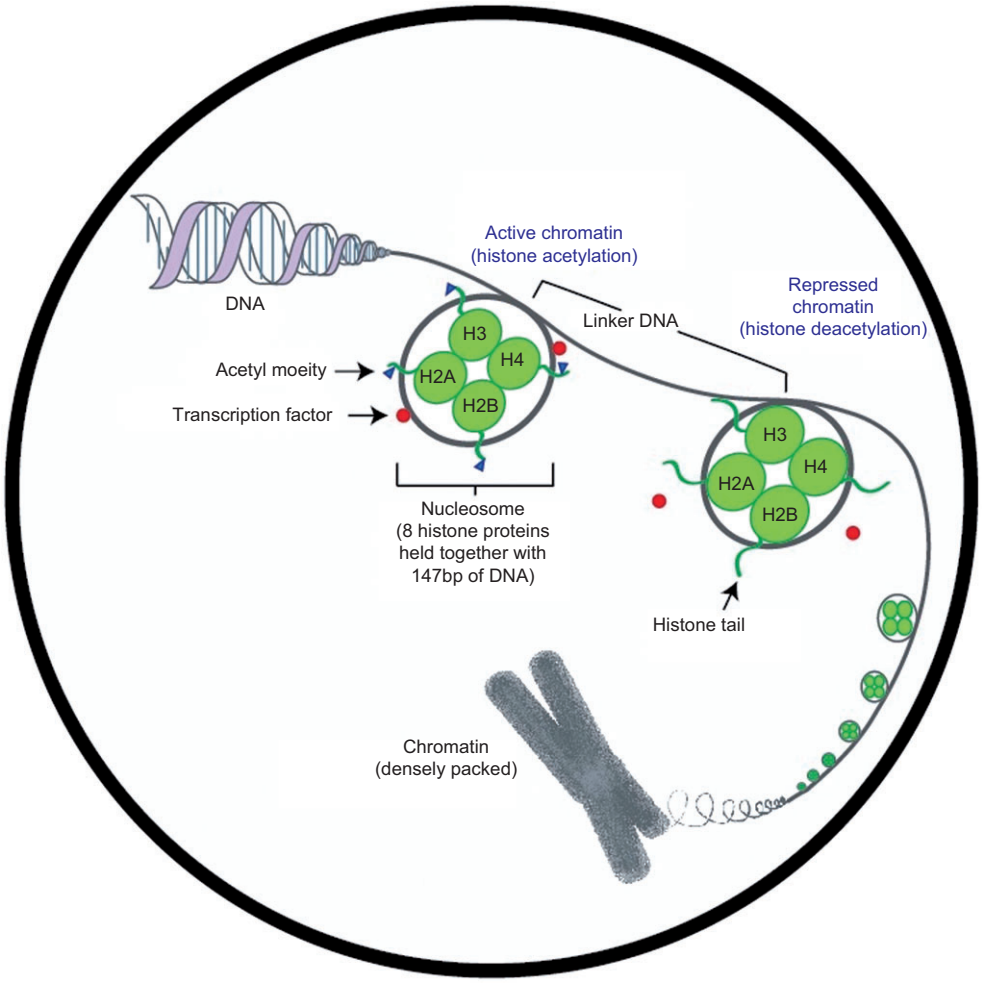


**Figure 1** Chromatin organization and histone acetylation.
The diagram depicts the organization of double-stranded DNA into a neatly packaged chromatin structure. The nucleosome comprises 147 bp of DNA wrapped around a histone octamer that undergoes PTMs. The acetylation of core histone tails promotes an active chromatin state, where DNA loosens from the histones and enables the transcription factors to interact with the DNA. Histone deacetylation initiates a repressed chromatin state, where the DNA tightens around the histones and prevents the transcription factors to associate with the DNA.

nonhistone substrates (45, 46) that fundamentally serve a higher order in transcriptional regulation. The maintenance of neurophysiological homeostasis is achieved by orchestrating a balanced HAT and HDAC interchange into a functional equilibrium, which ensures proper transcriptional regulation and gene expression (Figure 2) (4). During neurodegenerative maladies, the HAT and HDAC interplay is imbalanced where significant HDAC activity increases and HAT activity decreases (4, 47, 48). This results in the silencing of transcriptional activities, which is vital for proper neuronal functioning and survival. HDAC inhibitors, as their name implies, function to effectively elevate cellular acetylation and thus provide great therapeutic potential in the treatment of neurodegenerative diseases (7, 8). Some of the most recent and promising findings regarding epigenetic regulations in

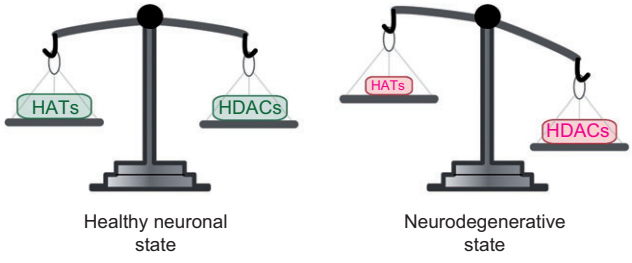


**Figure 2** Neuronal histone acetylation.
Weights on the balance symbolize the protein levels of HATs and HDACs in a healthy neuronal state and a neurodegenerative state. During normal neuronal physiological conditions, HATs and HDACs are at equilibrium, which they counteract each other to maintain an optimal balance of gene expression. In a neurodegenerative state, the histone acetylation balance is tilted where HDACs excessively outweigh HATs, which in turn silences the transcriptional processes, and subsequently, gene expression.

AD pathology and associated manifestations will be discussed in the following sections.

## Epigenetic mechanisms and AD-linked pathologies

One of the most profound pathological hallmarks associated with AD is the extracellular accumulation of amyloid plaques. The deposition of multiple Aβ fragments accumulate into a plaque through the proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. The β-secretase, commonly referred to as β-site APP-cleaving enzyme 1 (BACE1), initiates the defining PTM of the amyloidogenic pathway, which leads to AD pathogenesis (49). Recently, Marques et al. (50) discovered that *BACE1* mRNA levels and promoter accessibility were significantly increased in PBMCs in AD patients and progressively elevated in patients with MCI in comparison to that of healthy controls. Ultimately, this may provide a pattern in mapping crucial gene regulations involved in AD progression.

In addition, increased BACE1 mRNA expression has been shown to be associated with increased global H3 hyperacetylation (50, 51), which may be mediated *via* acetylation and deacetylation by p300/CREB-binding protein (CBP) and HDAC3, respectively (51, 52) (Table 1). Together with BACE1-mediated APP cleavage, these studies suggest that epigenetic mechanisms play a vanguard role in AD pathogenesis and progression, thereby regulating BACE1 expression and transcription. Moreover, these studies also refer to global H3 hyperacetylation as a potential early-stage biomarker during AD pathology. The examination of PBMCs have provided a reliable method to research epigenetic mechanisms, including histone acetylation, which may have arisen *via* external influences (23) and mirrored the aberrant epigenetic machinery present in the neurodegenerative brain (53). Current findings have also identified potential blood-based protein biomarkers

**Table 1** Role of HDACs and HATs during neurodegeneration.

| | Class | Role during neurodegeneration | References |
|---|---|---|---|
| HDACs | | | |
| 1 | 1 | Silences neprilysin gene expression at promoter regions | (60) |
| | | Promotes neuronal survival through restoration of double stranded DNA breaks *via* p25-mediated neurotoxicity | (108) |
| | | Complexes with HDAC3 and induces neurotoxicity | (110) |
| 2 | 1 | Negatively regulates memory functions in transgenic AD mice | (94, 96) |
| | | Silences neuroprotective genes implicated in learning memory and synaptic plasticity by inducing local chromatin compaction promoter regions | (94) |
| 3 | 1 | Negatively regulates memory formation | (97) |
| | | Selectively mediates apoptosis in neuronal cells | (109) |
| 5 | 2 | Regulates memory formation in transgenic AD mice | (91) |
| 6 | 2 | Exhibits increased expression in the AD brain and interacted with τ | (74) |
| | | Inhibition correlates with τ degradation | (74, 76) |
| | | Regulates mitochondrial movement in hippocampal neurons | (75) |
| | | Inhibition ameliorated dysfunctional mitochondrial movement induced by AB toxicity | (77) |
| 7 | 2 | Promotes neuronal survival *via* silencing c-jun at promoter regions | (107) |
| HDRP | 2 | Promotes neuronal survival by recruiting HDAC1 and silenced c-jun promoter regions | (111) |
| | | Elevated function reduces neurotoxic HDAC1-HDAC3 interaction | (110) |
| SIRT1 | 3 | Increases α-secretase transcription and expression | (66) |
| | | Activity correlates with τ ubiquitination | (68) |
| HATs | | | |
| CBP | | Enhances memory regulations | (83–86) |
| | | Selectively mediates memory consolidation | (84) |
| | | Depletion results in neuronal death and toxicity | (47, 99, 100) |
| | | Functions as a transcriptional co-activator to CREB | (101) |
| | | Over expression results in neuronal death within nutrient-rich conditions | (47) |
| PCAF | | Enhances memory formation | (89, 90) |
| | | Knockout mice exhibited resistance toward Aβ-mediated neurotoxicity | (106) |
| P300 | | Prevents phosphorylated τ degradation | (68) |
| | | Enhances memory consolidation | (87, 88) |
| P300/CBP | | Increases BACE1 mRNA expression at promoter regions | (51, 52) |

in circulation within AD patients (54–57), which, when verified, may offer advantages during clinical approaches particularly *via* the mildly invasive nature. Postmortem autopsy provides an accurate diagnosis of AD; therefore, early detection tests are crucial to attain an adequate diagnosis of AD and to monitor disease progression and potential treatments.

Following γ-secretase-mediated APP processing, the APP intracellular domain (AICD) is generated (49), which has been revealed to serve roles in transcriptional regulation (58, 59). Interestingly, this AICD domain has been shown to bind to neprilysin promoters and encourage *NEP* gene expression (60). Neprilysin is the most potent pathological Aβ-degrading enzyme (61), which has been shown to decrease and negatively correlate with both insoluble $A\beta_{40}$ and $A\beta_{42}$ deposition in the AD brain (62). Therefore, upregulating the neprilysin gene, and subsequently, the enzymatic activity, is a viable therapeutic strategy to promote pathogenic amyloid clearance in the AD brain. Belyaev et al. (60) showed that *NEP* promoters were enriched with H4K8 and H4K16 acetylation within a trancriptively active NB7 human neuroblastoma cell line. By contrast, the *NEP* promoter regions were occupied by the deacetylase HDAC1 within the transcriptively inactive SH-SY5Y human neuroblastoma cells. Treatment with HDAC inhibitors (Table 2), valproic acid (VPA) and trichostatin A (TSA), significantly increased *NEP* expression and enzymatic activity in SH-SY5Y cells but not in the NB7 cell line, suggesting Aβ clearance can be reinstated *via* chromatin remodeling. Chromatin immunoprecipitation analysis using anti-HDAC1 and anti-AICD antibodies revealed an increase in AICD binding to *NEP* promoter regions, which paralleled a decrease in HDAC1 binding within the SH-SY5Y cells after TSA treatment (60), which was also consistent with a subsequent *in vivo* study (63). These results indicate that HDAC1 silences *NEP* expression at promoter regions, and the downregulation exposes the *NEP* promoter and assists in AICD binding. In addition, intraperitoneal administration of VPA was shown to significantly increase neprilysin catalysis in the hippocampus of aged rats subjected to prenatal hypoxia, which corresponded with an enhancement of both short- and long-term memory formations (63). These studies identify HDAC1 as an attractive target to downregulate in order to facilitate the clearance of pathogenic Aβ in the AD brain and sequentially ameliorate the cognitive discrepancies associated with the neurodegenerative brain.

The non-amyloidic pathway commences with α-secretase-mediated cleavage of APP, which potentially decreases pathogenic plaque formation, thereby cleaving the APP between the regions of β- and γ-secretases (49). Recent evidence has emerged indicating that the deacetylase SIRT1 is highly neuroprotective during neurodegenerative diseases such as HD (64) and AD (65, 66). Of particular interest, SIRT1 mediates its neuroprotective roles by influencing APP processing, thereby increasing α-secretase transcription and expression (66). SIRT1-mediated deacetylation activates the retinoic acid receptor β, which associates with the *ADAM10* promoter regions and increases α-secretase transcription and production. As a result, SIRT1 deacetylase expression parallels with decreased Aβ plaque production in the brain (66). When

**Table 2** Role of HDAC inhibitors during neurodegenerative diseases.

| HDAC inhibitor | Role during neurodegenerative diseases | References |
|---|---|---|
| VPA | Inhibits HDAC1 and increases *NEP* expression *in vitro* | (60) |
| | Inhibits HDAC1 and increases NEP expression and enzymatic activity hypoxic rodents | (63) |
| | Enhances short- and long-term memory formation in hypoxic rodents | (63) |
| | Prevents Aβ deposition in transgenic AD mice | (78) |
| | Promotes the expression of BDNF and GDNF transcripts in neuronal cells within neurotoxic conditions | (105) |
| TSA | Increases *NEP* expression in neuroblastoma cell lines | (60) |
| | Promotes the expression of BDNF and GDNF transcripts in neuronal cells within neurotoxic conditions | (105) |
| TBA | Exhibits HDAC6 selectivity and restores dysfunctional mitochondrial movement induced by Aβ toxicity | (77) |
| PBA | Reduces NFT and Aβ pathology in transgenic AD mice | (79, 80) |
| | Enhances cognitive functions and memory deficits in transgenic AD mice | (79, 80) |
| | Increases the expression of synaptic plasticity markers | (79) |
| | Restores dendrite spine defects in transgenic AD mice | (80) |
| W2 | Exhibits HDAC class 2 inhibitory functions | (81) |
| | Increases the expression of Aβ degrading enzyme Mmp2 | (81) |
| SAHA | Promotes the expression of BDNF and GDNF transcripts in neuronal cells within neurotoxic conditions | (105) |
| RGFP123 | Exhibits HDAC3 selectivity and enhanced memory formation within transgenic mice | (97) |
| NaBu | Enhances memory functions in transgenic AD mice | (48) |

considering therapeutic treatments for AD, SIRT1 expression should be considered a potential method of deterring the formation of pathogenic Aβ production by influencing the α-secretase-mediated APP cleavage toward the non-amyloid pathway.

In the AD brain, the microtubule-associated protein τ undergoes hyperphosphorylation, forming intracellular NFT, which leads to increased neuronal dystrophy and progressive deterioration of the brain (67). Recently, research has revealed that τ acetylation is a critical PTM associated with τ-mediated neurodegeneration (68–70). τ Acetylation advances in the AD brain in response to Aβ pathologies and is increased in patients during mild to moderate Braak stages (68). It was also discovered that increased p300-mediated τ acetylation prevents phosphorylated τ degradation, whereas increased deacetylation activity of SIRT1 parallels with τ ubiquitination (68). Notably, increased τ acetylation at K280 provokes τ polymerization into pathological NFT formation, accelerating tauopathological neurodegeneration (69, 70). Taken together, these studies indicate that tauopathological-mediated AD may therefore be considered a neurodegenerative disease as characterized by hyperacetylation activities.

In neurodegenerative diseases, the activities of the deacetylase HDAC6 are still relatively unknown, where its activity has shown to be desirable within PD (71, 72) but detrimental to HD (73) and AD (74, 75). More specifically, HDAC6 exhibits increased expression in the AD brain (52% in the cortex and 91% in the hippocampus) and interacts with τ (74). The inhibition of HDAC6 correlates with τ degradation (74, 76) and regulates mitochondrial movement in hippocampal neurons (75). In addition, tubastatin A (TBA), a HDAC6-specific inhibitor, ameliorated the dysfunctional mitochondrial movement induced by Aβ toxicity (77). Taken together, these studies demonstrate that HDAC6 is a desirable target to downregulate in the context of AD.

HDAC inhibitors have shown great potential in alleviating neurodegenerative manifestations even at very advanced stages of AD (48). For example, the HDAC inhibitor VPA significantly prevents Aβ plaque deposition in the brains of transgenic AD mouse models by preventing APP γ-secretase cleavage *via* glycogen synthase kinase-β3-dependent mechanisms (78). Sodium 4-phenylbutyrate (PBA) was shown to reduce hyperphosphorylated τ pathology (79) and facilitate Aβ clearance in the brain of an AD transgenic mouse model (80). In addition, W2, a novel mercaptoacetamide-based class 2 HDAC inhibitor, was shown to downregulate the expression of γ-secretase counterparts and upregulate the expression of Aβ degrading enzyme matrix metallopeptidase 2 (Mmp2) in a transgenic AD mouse model (81). On a phenotypic basis, W2-administered mice exhibited a decrease in both $A\beta_{40}$ and $A\beta_{42}$ plaque levels and phosphorylated τ at Thr181 in comparison to control littermates. This study points to a functional role that class 2 HDACs may serve during the pathogenesis of AD (81). Taken together, these studies demonstrate that HDAC inhibitors provide promising therapeutic potentials in ameliorating AD-related pathologies.

## Epigenetic mechanisms and cognitive performances

Progressive memory loss is one of the most prominent clinical symptoms associated with AD, and age is a great predisposing factor. Interestingly, Peleg et al. (82) discovered that altered histone acetylation correlates with age-dependent learning and memory impairment. Both young and aged mice display increased H3K9, H3K14, H4K5, and H4K8 acetylation 1 h after fear conditioning. However, the aged mice failed to increase H4K12 acetylation in comparison to the young mice, suggesting that memory impairment is associated with defective acetylation of H4K12. Furthermore, hippocampal administration of the HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), increased H4K12 acetylation, which was also increased at the promoter regions of learning-regulated genes (82). This study successfully demonstrated that impaired memory regulation processes during brain aging can be maintained through chromatin remodeling.

Histone acetylation results in a transcriptionally active chromatin, which in turn facilitates gene expression. The functions of the HAT CBP in the nervous system are highly regarded due to their fundamental roles during memory regulations (83–86). A recent study conducted by Barrett et al. (84) revealed that CBP selectively mediates memory consolidation. This study generated focal homozygous *Cbp* deletion in the hippocampal CA1 subregion in mice, which displayed long-term memory deficits in contextual fear and object location paradigms. Immunohistochemical analysis revealed the H2BK12, H3K14, and H4K8 acetylation was significantly reduced in hippocampal neurons lacking *Cbp*. CBP upstream signaling occurred, as CREB phosphorylation was evident; however, CREB:CBP-mediated *c-fos* gene expression was not achieved, indicating that CBP functions are central for CREB:CBP-coupled gene expression. Interestingly, systemic administration of HDAC inhibitor sodium butyrate (NaBu) failed to alleviate memory deficits. This is a strong indication that CBP functionality is vital for

long-term memory formation, as CBP loss cannot be compensated by its sister HAT, p300 (84). Similarly, p300 provides beneficial entities during memory consolidation, as transgenic mice containing defective p300 (87) and subregion-specific p300 knockout (88) were shown to exhibit long-term memory deficits. The results of this study strongly indicated that the loss of p300 cannot be compensated by CBP (88), further highlighting the imperative functionality these acetyltransferases mediate during memory regulations. Additionally, p300/CBP-associated factor (PCAF) functions have shown an involvement during memory formation as an upregulation correlates with fear extinction memory (89) and PCAF knockout results in memory impairment (90).

In addition to HAT activity, recent evidence has emerged that suggests HDAC5 is vital for memory functions during AD-mediated neurodegeneration (91). This research used four experimental groups of rodents (including wild type) established from a C57BL/6J genetic background. The AD AβPP/PS1-21 (AβPP) rodent model encompasses a double transgene of mutated APP (KM670/671NL) and presenilin 1 (L166 P) influenced by a neuron-specific promoter, Thy1, which exhibits cerebral Aβ plaque pathology as early as 6 weeks old (92). The authors also used HDAC5 knockout mice (HDAC5$^{-/-}$) and cross-bred them with AβPP rodents, generating offspring exhibiting the AD double transgene and HDAC5 depletion (AβPP-HDAC5$^{-/-}$) (91). It was observed that spatial memory performance was significantly impaired in the Aβ-PPHDAC5$^{-/-}$ mice, which was comparable to the performance of HDAC5$^{-/-}$ mice, in comparison to that of both the AβPP and wild-type mice. Rodents were also fear-conditioned to examine associative memory functions among the four groups. The analysis of tone-dependent memory consolidation revealed a significant decrease in the freezing behavior of AβPP-HDAC5$^{-/-}$ and HDAC5$^{-/-}$ mice in comparison to AβPP and wild-type mice (91). This indicated that a loss of HDAC5 significantly impairs memory consolidation greater than AD-mediated pathology alone. Therefore, when considering the therapeutic treatment of cognitive decline during AD-mediated neurodegeneration, HDAC5 inhibition should be avoided.

A pioneering study revealed that the nonselective HDAC inhibitors SAHA, NaBu, and VPA inhibited class 1 HDACs with greater efficiency than class 2 isoforms in an AD mouse model exhibiting memory deficits (93). This strongly suggests that class 1 HDACs are central for treating memory impairments seen in AD. In addition, HDAC2 has been identified as an epigenetic blockade of cognitive function seen in the AD brain (94). Using a transgenic CK-p25 mouse model overexpressing p25 within the forebrain (95), it was shown that HDAC2 silences the neuroprotective genes that are implicated in learning, memory, and synaptic plasticity by inducing local chromatin compaction at promoter regions (94). This suggests that cognitive decline during neurodegenerative disease is negatively mediated by HDAC2 functionality, which is consistent with earlier findings (96). The authors of the recent study discovered that the presence of AD-related neurotoxic stimuli, $A\beta_{1-42}$ oligomers, increased *Hdac2* mRNA and transcription, which were found to be mediated *via* the activation of the transcription factor glucocorticoid receptor 1. Reducing the HDAC2 levels using adeno-associated viral vectors bearing short-hairpin RNA resulted in H4K12 hyperacetylation at promoter regions of neuroprotective genes, which ameliorated the synaptic plasticity and neuronal morphology. This indicates that during Aβ-mediated neurodegeneration, HDAC2 induces cognitive dysfunction by silencing neuroprotective genes at their promoter regions (94). These studies demonstrate the significance of an HDAC2-specific inhibitor for the therapeutic treatment of cognitive disorders associated with neurodegenerative diseases.

In addition, McQuown et al. (97) indicated that HDAC3, the most highly expressed class 1 HDAC in the brain, has a critical role in the molecular mechanisms that underlie long-term memory formation (42). In this study, adeno-associated virus-expressing Cre recombinase was used in conjunction with HDAC-FLOX genetically modified mice in an attempt to produce focal homozygous deletions of HDAC3 in the dorsal hippocampus. A pharmacological approach was used in conjunction with the genetic study where the delivery of RGFP123, a selective inhibitor of HDAC3, was shown to cause an increase in histone acetylation within the hippocampus and a consequent enhancement of long-term memory function. Expressions of the nuclear receptor Nr4a2 and the protein *c-fos* were significantly higher in the hippocampus of the genetically modified mice than in controls, indicating their integral involvement in memory formation. Further research indicated that when *Nr4a2* was inserted into the hippocampus of the HDAC3-FLOX mice, the memory formation enhancements were abolished, strongly indicating a negative regulation of memory formation by HDAC3. The genetic and pharmacological approaches of this study provided a strong support for the integral role of HDAC3 in the underlying mechanisms that drive long-term memory formation (97).

The HDAC inhibitor NaBu has shown great therapeutic promise in ameliorating cognitive discrepancies in mice at advanced stages of AD (48). This study used aged AβPP transgenic mice and assessed the associative

memory function following intraperitoneal injection of NaBu or vehicle treatments. NaBu-administered mice displayed significantly increased freezing behavior during the Pavlovian fear conditioning paradigm in comparison to vehicle-treated mice. This suggests that NaBu facilitates associative learning behavior in advanced stages of AD pathology (48). PBA was shown to enhance associative learning in both aged wild-type mice and aged transgenic AD mice (80). Furthermore, PBA administered to aged Tg2576 mice expressing the human 695-amino acid isoform of APP containing the Swedish double mutation and exhibiting accelerated AD pathology improved the spatial memory defects, recovered the loss of H4 acetylation, and promoted the expression of synaptic plasticity markers (79). Taken together, these studies demonstrate that HDAC inhibitors could provide an effective method of treating cognitive disorders seen in AD.

## Epigenetic mechanisms and neuronal protection

Postmitotic neurons do not divide and persist throughout the lifetime of an individual. Neuronal cell degradation and death is a characteristic feature of neurodegenerative diseases, including AD. Neuronal apoptotic events have been shown to follow H3 and H4 hypoacetylation (47), which is indicative of a repressive chromatin state. Therefore, a balanced HAT and HDAC activity is vital for neuronal survival by appropriately regulating the transcriptional factors and actively promoting and silencing gene expression, respectively (4). In the nervous system, transcriptional dysregulation leads to such manifestations that can occur through the upregulation of death-inducing genes and silencing genes involved in neuronal survival (10, 47, 98).

In addition to memory regulation, the acetyltransferase CBP is also a beneficial regulator of neuronal cell viability. This has been made evident by a loss of its function, which consequently results in neuronal cell death and toxicity during neurodegenerative maladies (47, 99, 100). Additionally, CBP functions are highly regarded in part due to its influence as a coactivator to the transcription factor CREB (101). CREB functionality within the brain poses high significance, as a previous study revealed that a disruption of CREB leads to neuronal apoptosis and progressive neurodegeneration of the mouse brain (102). It has also been shown that an upregulation of wild-type CREB (in comparison to mutated CREB) significantly reverses the incidence of neuronal apoptosis within cytotoxic conditions, thereby increasing the gene expression of the vital neurotrophin brain-derived neurotrophic factor (BDNF) and activity-regulated inhibitor of death genes (103). Attenuating the chromatin state with the use of HDAC inhibitors can remarkably reverse neuronal apoptotic death (98, 104). HDAC inhibitors VPA, TSA, and SAHA induced the expression of neuroprotective transcripts BDNF and glial cell line-derived neurotrophic factor (GDNF) *via* H3 hyperacetylation at promoter regions in dopaminergic neurons in the presence of neurotoxin $MPP^+$ (105). Furthermore, PBA treatment ameliorates dendrite spine deficits in Tg2576 hippocampal neurons (80).

An elevated level of HAT activity is a favorable entity in neuronal cells; however, it has been discovered that CBP overexpression in nutrient-rich conditions can lead to apoptotic morphology in neuronal cells (47). This provides great insight regarding the fundamental regulatory processes of HATs and HDACs in neuronal survival. Although CBP functionality is highly neuroprotective (99, 100), its upregulation is neurotoxic, suggesting that fine-tuning HAT activities is a vital mechanism to augment neuronal survival during both healthy and neurodegenerative conditions (47). In addition, PCAF knockout mice have exhibited resistance toward $A\beta_{25-35}$-mediated neurotoxicity (106). Rodents received intracerebroventricular administration of $A\beta_{25-35}$, and their hippocampi were removed 7 days following treatment. The expression analysis of oxidative stress marker (peroxidized lipids), cellular stress marker (caspase 3), and endoplasmic reticulum stress marker (caspase 12) showed significant increased expression in the hippocampal CA1 regions of wild-type littermates in comparison to the PCAF knockout mice (106). This finding suggests that a downregulation of PCAF activities may provide a positive mechanism in ameliorating Aβ-mediated neurodegenerative manifestation, thereby weakening the proapoptotic mediators.

Moreover, previous studies have discovered that nonspecific HDAC inhibitors induce neuronal apoptosis under prosurvival conditions (9, 10). Collectively, increasing histone hyperacetylation activity leads to a transcriptional active chromatin state where proapoptotic mediators can be expressed (10). It is therefore reasonable to conclude that certain HDAC isoforms are required to selectively repress transcriptional processes, which in turn mediate neuronal survival. For example, it was thought that HDAC7 activities are highly neuroprotective during neurodegeneration (107). It was shown that HDAC7 translocates into the nucleus where it arbitrates its neuroprotective effects, thereby inhibiting the transcription of proapoptotic mediator c-*Jun via* the interaction with its promoter. It was also demonstrated that forced HDAC7

expression reversed neuronal cell death in the presence of neurotoxic Aβ peptides in rodent cortical neurons (107). There are several beneficial activities associated with HDAC7 expression, particularly increasing the survival of neurons during Aβ-mediated neurodegeneration seen in AD. HDAC1 functions have also been associated with neuronal survival through the restoration of double-stranded DNA breaks *via* p25-mediated neurotoxicity (108).

In a contrast, HDAC3 has been demonstrated to strongly influence neuronal death (109). The effects of forced HDAC3 expression was analyzed in rodent cerebral granule neurons under prosurvival (i.e., high potassium) and proapoptotic (i.e., low potassium) conditions, which resulted in neuronal loss and complete neurodegeneration, respectively. Further analysis using TUNEL staining confirmed HDAC3 toxicity, whereas the presence of caspase 3 was confirmed *via* immunohistochemistry. This effect was trialed with primary kidney fibroblasts, HEK293 and HeLa cell lines, where no effect of cellular viability was evident, suggesting that HDAC3-mediated toxicity is selective to neuronal cells. Additional research using two separate short-hairpin RNA constructs against HDAC3 protected the cerebellar granule neurons and cortical neurons from death initiated by apoptotic stimuli. Taken together, this study effectively identified HDAC3 as a potent neurotoxic deacetylase and therefore becomes an attractive target to downregulate in an effort to increase neuronal viability during neurodegeneration (109). In a subsequent study, the authors showed that HDAC1 contributes to neurodegeneration and is highly neurotoxic, as it complexes with HDAC3 in mediating neuronal death (110). Previously, the HDAC-related protein (HDRP), a truncated form of HDAC9, was shown to recruit HDAC1 and deacetylate H3 at *c-Jun* promoter regions, which is dependent upon HDAC1 deacetylase activity (111). Bardai et al. (110) showed that HDAC1 and HDRP associate *via* HDAC1 *N*-terminal tails and the neurotoxic HDAC1-HDAC3 interaction is reduced when HDRP expression is elevated, suggesting that HDAC1 is sequestered by HDRP. Taken together, HDAC1 performs as a molecular switch in regulating neuronal fate. When HDAC1 and HDAC3 interact, they work in tandem in mediating neuronal death; however, HDAC1 functions are neuroprotective when HDRP associates with and interferes with HDAC1-mediated neurotoxicity (110).

Findings have surfaced suggesting that excessive zinc in neuronal cells is toxic, thereby, influencing Aβ aggregation in the brains of transgenic AD mice (112) and colocalizes with Aβ plaques in the brains of AD patients (113). In contrast, DHA has shown neuroprotective properties, as high levels of dietary DHA protected against Aβ neurotoxicity and decreased plaque load in AD transgenic mice (114). On an epigenetic basis, our research group discovered that zinc and DHA can directly influence the expression levels of H3 and H4 in M17 human neuroblastoma cells. The zinc effect (absence of DHA) resulted in a downregulation of H3 and H4 mRNA. However, in the DHA effect (presence of zinc and DHA), histone expression was considerably upregulated (115). In a subsequent study, Sadli et al. (25) looked at the effects of zinc and DHA on the acetylation level of H3K9. It was discovered that H3K9 was significantly hypoacetylated with zinc and hyperacetylated with DHA. Further research demonstrated that zinc and DHA have opposite effects in the modulation of HDAC1, HDAC2, and HDAC3 expression levels. HDAC1, HDAC2, and HDAC3 were significantly increased during the zinc effect and were significantly decreased during DHA effect, a dysregulated acetylation balance characteristic of neurodegeneration (4). Furthermore, during the zinc effect, caspase 3 expression increased, whereas Bcl-2 expression decreased. This observation was in complete contrast to the DHA effect, which demonstrates the highly neuroprotective properties that DHA offers in modulating the chromatin state and thus ameliorating the incidence of neurodegenerative diseases (25). This research opens up new avenues to therapeutic potentials in treating neurodegenerative diseases such as AD.

## Expert opinion

A balanced HAT and HDAC interplay is vital for neuronal cell functionality, thereby coordinating gene expression in a healthy manner. During a neurodegenerative state, this balance is pushed where HDAC activity overrides HAT activity, resulting in a repressed chromatin state (4). Previous studies have shown the beneficial functions that pan-HDAC inhibitors can offer in the treatment of neurodegenerative diseases; however, there have been reports of contradictory effects (9, 10, 116). Knowledge of individual HAT and HDAC functions in the nervous system will prove beneficial in designing appropriate drug targets to ameliorate the incidence of neurodegenerative diseases. Research has flourished in recent years and has shown that certain HDAC isoforms are relatively neuroprotective (66, 91, 107, 108) and inhibition should be avoided. Other HDAC isoforms have shown to selectively mediate neurotoxic effects (94, 97, 109), and focus should be put on efforts to downregulate these specific isoforms. Additionally, certain HDACs have shown to mediate differential roles during neurodegenerative diseases (71–75, 77, 108, 110). Therefore, more research is required to decipher

the precise mechanism by which certain HDAC isoforms mediate neurotoxic effects and manufacture inhibitors with great specificity in the context of the neurodegenerative disease. Additionally, isoform specificity will aid in augmenting the acetylation balance into a desirable neurophysiological state. Pan-HDAC inhibition within prosurvival conditions resulted in neuronal catastrophes (9, 10); thus, a balance of HDAC functionality is required to augment the chromatin state.

## Outlook

Isoform-specific inhibitors have a great potential in the treatment of neurodegenerative diseases. Combined with the knowledge of individual HDAC function and influences in the nervous system, isoform-specific inhibitors will allow for the downregulation of exclusively neurotoxic HDACs. Recent research strongly indicates that isoform-specific inhibition could provide a promising prophylactic treatment for neurological disorders.

## Highlights

- SIRT1 activity promotes the expression of α-secretase by activating the retinoic acid receptor β, which associates with the ADAM10 promoter regions and increases the α-secretase expression. Therefore, SIRT1 functionality correlates with reduced Aβ plaque formation.
- HDAC6 inhibition correlates with τ degradation and ameliorated the dysfunctional mitochondrial motility induced by Aβ toxicity, pointing to fundamental properties of HDAC6 may provide to alleviate neurodegenerative manifestations.
- Loss of HDAC5 functionality in the AD brain significantly impairs memory function; therefore, therapeutics should be aimed at upregulating HDAC5 to treat cognitive disorders associated with neurodegeneration.
- HDAC2 functions as an epigenetic blockade by silencing the neuroplasticity genes at the promoter regions in transgenic AD mouse brain; thus, great efforts are needed to manufacture a promising HDAC2-specific inhibitor to effectively alleviate memory deficits associated with AD.
- HDAC3, a negative regulator of memory formation, is an attractive target to reduce in order to facilitate adequate memory functions.
- PCAF knockout mice exhibit resistance to Aβ toxicity, signifying that a downregulation of PCAF may have beneficial entities in remedying AD.
- HDAC7 is a neuroprotective deacetylase, as it interacts and silences c-*Jun* expression during Aβ-induced neurotoxicity; therefore, its expression should be encouraged to sustain neuronal integrity within AD patients.
- An increased expression HDAC3 selectively induces apoptosis and toxicity in neuronal cells, and for this reason, this specific isoform is an attractive target to inhibit when treating neurodegenerative diseases and other neuronal disorders.

**Conflict of interest statement:** The authors declare that there is no conflict of interest.

Received December 4, 2012; accepted February 26, 2013

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